Committee:
Reimann, Chm.
Kotin
Jacobson

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-second Street
New York 17, N. Y.

#271

TIRC file

Application for Research Grant

Date: March 4, 1960

1. Name of Investigator: P. O'B. Montgomery, Jr., M.D.

2. Title: Associate Professor of Pathology, The University of Texas Southwestern Medical School.

3. Institution & Address: The University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas 35, Texas

4. Project or Subject: The Investigation of the Role of the Nucleolus in the Normal and the Malignant Cell

Beginning Date: May 1, 1960 to April 30, 1961

5. Detailed Plan of Procedure:

The purpose of this study will be to investigate the role of nucleolar function in living normal and malignant cells. The principal technics for study will be time-lapse motion pictures of living cells as demonstrated by phase contrast, interference and ultraviolet microscopy. Correlative technics used will be electron microscopy, fluorescence microscopy and histochemistry.

The nucleolus is the only morphologic entity which can be resolved in the living cell whose function is obscure. Its enlargement and duplication in malignant cells, its intimate association with mitosis, and its hypertrophy in response to carcinogenic chemicals and viruses; all point to it as a structure with a key role in the transformation of normal to malignant cells. The main feature which the technics used in this study will bring to bear on the problem is elucidation of the temporal relationships between the nucleolus and the cell's structure, function and chemistry.

The most direct biological approach to this problem consists in the removal of the nucleolus of one living cell and its introduction into the cytoplam of a second living cell. Preliminary experiments in this laboratory have demonstrated that nucleoli may be removed from one living cell and transplanted to the cytoplasm of a second living cell. Phase contrast time-lapse motion picture observations of such transplanted nucleoli show that they frequently initiate mitosis in the recipient cell. In those recipient cells in which mitosis is not initiated the foreign nucleolus is brought to a juxtanuclear position and there eventually undergoes dissolution. These preliminary experiments will be continued and amplified in

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the following three directions. One direction will be the transplantation of nucleoli from malignant cells into benign cells of a similar strain. The second and third directions will consist of the recording of the results of nucleolar transplantation in homologous cells and in heterologous cells by means of time-lapse motion picture photography using interference and ultraviolet microscopy. These latter two studies are designed to elucidate the role of the nucleic acids of the transplanted nucleolus in the recipient cell and the relationship of the transplanted nucleolus to the dry protein mass of the recipient cell.

The second major technic to be applied to the investigation of the role of the nucleolus in cell function will be ultraviolet microbeam irradiation of the nucleolus of the living benign and malignant cell. For this experimental approach the ultraviolet flying spot television microscope is the most elegant tool yet devised. This instrument is capable of producing a microbeam of ultraviolet down to 1 micron square. The microbeam may be visually positioned in size and shape so that the nucleolus of the cell is continuously subjected to ultraviolet irradiation, while the remainder of the cell and the field is illuminated by visible light. The results of these observations may then be recorded by time-lapse motion picture photography. At regular intervals during the course of the experiment ultraviolet absorption images of the entire cell may be obtained, along with quantitative analyses of the density of the nucleolar absorption of ultraviolet light. These experiments are designed to add to our information on the relationship between nucleolar RNA and cytoplasmic RNA in benign and malignant cells. A complete description of the technical aspects of the ultraviolet flying spot television microscope may be found in the references of the accompanying bibliography.

The third experimental procedure to be used in the investigation of the role of the nucleolus in cell physiology will be the influence of carcinogenic compounds on the nucleolus. Drs. Endo, Aoki and Aoyama of the Japanese Cancer Institute have recently described the production of nuclear inclusions in tissue culture cells treated with the carcinogenic compound 4-Nitroquinoline N-Oxide. Through the kindness of Dr. Endo we have been provided with a supply of this compound. Preliminary experiments in this laboratory have confirmed their observations. (Gann 50, 209-217, Sept. 1959) These observations will be extended in this laboratory to elucidate the role of the nucleolus in formation of the nuclear inclusions. The development of the inclusions will be recorded by time-lapse motion picture photography with phase contrast, interference and ultraviolet microscopy. The chemical composition of the inclusions will be contrasted with that of the nucleolus by histochemical procedures. The ultra-structure of the inclusions and the nucleoli will be compared by electron microscopy.

This series of experiments on the role of the nucleolus in cell function is expected to continue for approximately five years.

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6. Budget Plan: (See attached sheet)

Salaries	$3,113.00
Expendable Supplies	7,000.00
Permanent Equipment	3,000.00
Overhead (15%)	2,117.00
Other	1,000.00
Total	$16,230.00

Source: https://www.industrydocuments.ucsf.edu/docs/hjvm0000

7. Anticipated Duration of Work: Five years

8. Facilities and Staff Available:

Staff: Tissue culture technician full time; laboratory aid part time; senior electronics engineer full time; assistant electronics engineer full time; Resident in Pathology supported by investigative fellowship from American Cancer Society, eight medical student assistants part time.

Facilities: Ultraviolet flying spot television microscope, micromanipulator; two phase contrast time-lapse motion picture units; one time-lapse interference motion picture unit; complete tissue culture laboratory. All of the above are available full time under the senior investigator's direction - Available in the Department of Pathology on a part time basis are: a fluorescence microscope; electron microscope and complete histochemical laboratory with their associated personnel of three full time people.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The presently constituted laboratory is supported jointly by the Damon Runyon Memorial Fund for Cancer Research, the Atomic Energy Commission-Division of Biology and Medicine, and the Southwestern Medical Foundation. The over-all objectives of the laboratory are the development and application of new technics for the study of living cell physiology. The principal efforts are directed towards the introduction of temporal resolution into living biological systems in an effort to determine the functioning role of the nucleic acids in relationship to cell structure. Experiments designed to elucidate these points utilize ultraviolet irradiation, virus inoculation, and the introduction of specific carcinogenic chemicals into living cells. The results are recorded by ultraviolet time-lapse motion picture photography, phase contrast time-lapse motion picture photography, and interference time-lapse motion picture photography. Control studies of fixed material are carried out with electron microscopy, fluorescence microscopy and histochemistry.

/s./ P. O'B. Montgomery M. D.
Director of Project

/s./ E. A. Gell
Business Officer of the Institution
5323 Harry Hines Blvd.
Dallas 35, Texas

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6. Budget Plan:

Salaries:	
Student Laboratory Assistants (hourly)	$ 3,000
OASI and WCI	113
Expendable Supplies:	
Tissue culture supplies, glassware, biological supplies, film, electronic supplies, scanner tubes, chemicals, enzyme	7,000
Permanent Equipment:	
Optical equipment, electronic components, power supplies, timers, camera equipment	3,000
Other:	
Travel - For visitation to other laboratories, for attendance at scientific meetings	1,000
	$14,113
Overhead:	
15% of Direct Costs	2,117
TOTAL	$16,230

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